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Using *Duddingtonia flagrans* in calves under an organic milk farm production system in the Mexican tropics

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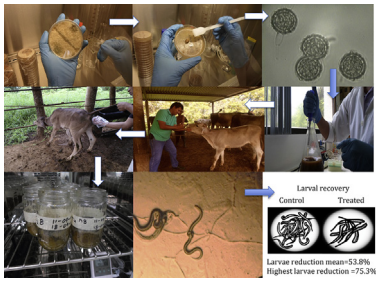
HIGHLIGHTS

- A method of bio-control of cattle nematodes in an organic milk farm was assessed.
- *Duddingtonia flagrans* chlamydospores were orally administered in grazing calves.
- Fungi reduced 53.8% (average) and 75.3% (highest) the GIN faecal larvae population.

GRAPHICAL ABSTRACT



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ABSTRACT

The reduction of the gastrointestinal nematode (GIN) larvae population in faeces of cattle treated with *Duddingtonia flagrans* chlamydospores on a farm under an organic production system in Chiapas, Mexico, was assessed. Seventeen Cebu/Swiss crossbreed grazing calves naturally infected with GIN, were randomly distributed into two groups and treated as follows: Group 1, an oral administration of 2×10^6 *D. flagrans* chlamydospores/kg BW, every two days for 30 days; group 2, Control, without any treatment. Results indicated that the epg values in both groups remained similar ($p > 0.05$). The average number of (L_3) from coprocultures from the group treated with *D. flagrans* had an important reduction (53.8%) with respect to the control group and it reached 75.3% maximum larval reduction at the 14th sampling; although, no statistic significance was observed ($p > 0.05$). Likewise, the average of larvae (L3) recovered from grass corresponding to the animals treated with *D. flagrans* diminished at 25.1% with respect to the control group ($p > 0.05$). A mixture of GIN genera including *Strongyloides* sp., *Haemonchus* sp., *Cooperia* sp., *Trichostrongylus* sp., *Oesophagostomum* sp. and *Mecistocirrus* sp., were identified from coprocultures. It was concluded that treatment with *D. flagrans* chlamydospores reduces the GIN larvae population in grass and in faeces of calves maintained under an organic milk production system.

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1. Introduction

The organic milk production systems require special attention in the management of a number of infective diseases, particularly the gastrointestinal parasitic nematodosis (GIN) (Barbosa-da Silva et al., 2012). The use of chemical anthelmintic drugs in organic production systems is restricted by the normativity and only two deworming (orally) treatments a year are permitted and only when it is absolutely necessary in terms of health and welfare risk. Deworming of animals for slaughter is banned (Nahed et al., 2008). So, sustainable alternatives of control of GIN need to be established in order to facilitate the requirements required by the law for organic milk production. Biological control of GIN using nematophagous fungi has been proposed as a clean alternative to control nematode parasitic infections (Da Silva et al., 2014). The use of nematophagous fungi is based on the fact that these organisms are able to form trapping devices especially designed to trap nematodes and destroy them and eventually feed on the nematodes inner tissues (Mendoza-de Gives and Torres-Acosta, 2012). The species *Duddingtonia flagrans* has been considered as the most promising nematophagous fungus for controlling GIN in calves and small ruminants worldwide (Assis et al., 2012, 2013; Wang et al., 2015). Likewise, the frequent administration of chlamydospores of this species has achieved good results in the control of parasitic nematodes in small ruminants (Paraud et al., 2005). This research was aimed to assess the effect of using *D. flagrans* chlamydospore in reducing the GIN larval population in faeces of calves maintained under an organic milk production system in Chiapas, Mexico.

2. Materials and methods

2.1. Allocation

This study was performed at the units of organic milk production in Mezcalapa municipality, Chiapas, Mexico, which is located in southern Mexico and at northeast Chiapas state between 94° 05′and 91° 23′ West longitude and between 17° 16′ North longitude. Weather conditions in Mezcalapa municipality are warm and humid with rainy summers (Aw) (69.54%). It is warm and humid with rain during the entire year (Af) (30.46%) with 1932 mm annual rainfall and is 320 m above sea level (Kottek et al., 2006).

2.2. Duddingtonia flagrans chlamydospore production

The FTHO-8 strain of *D. flagrans* was used. Chlamydospore production was performed at the laboratory of Helminthology of the National Centre for Disciplinary Research in Veterinary Parasitology (CENID-Parasitología Veterinaria, INIFAP-Mexico) in Jiutepec municipality, State of Morelos, Mexico. Chlamydospore production was achieved by growing fungus in potato dextrose agar plates followed by a 30 day incubation period at room temperature 25 to 29 °C. Chlamydospores were collected by adding distilled water and scrapping the agar surface and eventually collecting the resulting chlamydospore suspension in 1000 ml flasks. Chlamydospores were counted and kept at 4 °C until use.

2.3. Animals

Fifty American brown Swiss zebu male and female calves from 8 to 12 months of age naturally infected with GIN were randomly sampled from rectum to collect fresh faeces. Animals had been grazing on African star grass (*Cynodon nlemfluencis*) and Insurgente grass (*Brachiaria brizantha*). The number of GIN eggs eliminated per g of faeces was estimated using the McMaster technique (Thiempont et al., 1982) and seventeen calves with 500 epg values or higher were selected for this study.

2.4. Experimental

Selected animals were randomly distributed into 2 experimental groups as follows: Group 1 (n = 9 calves) received an oral treatment with an aqueous suspension containing *D. flagrans* chlamydospores at a dose of 2×10^6 chlamydospore per Kg Body Weigh (BW). Treatments were established every two days for 30 days. Group 2 (n = 8 calves) was considered a negative group without any treatment. During the experiment animals of the two experimental groups remained under the same grazing system than before the experiment in individual grazing areas designed for each group. No concentrated food was supplied and only mineral salts were offered them. Animals were allowed to graze (on the same pasturages previously described) from 6:00 in the morning, until 13:00 in the afternoon. After grazing animals were allocated into individual pens per group (per treatment). Fresh faeces from every animal were collected directly from rectum every two days after starting the treatment day and individual faecal cultures were prepared to obtain GIN infective larvae (L3) (Liébano-Hernández et al., 2011). Grass samples from surrounding fresh faecal matter depositions were collected every third day to obtain, quantify and identify GIN larvae. The GIN larvae either from faecal cultures or from grass were recovered through the Baermann funnel's technique. The number of larvae was estimated by counting the number of larvae in ten 5 μL aliquots of the larval suspension (Fitz-Aranda et al., 2015). The nematode larvae taxonomic identification was based on the guide for larval morphological identification published by Van-Wyk and Mayhew (2013). Viability of fungal chlamydospores after passing the gastrointestinal tract of calves was visualized under the microscope after depositing one faecal pellet of the experimental animals on the surface of water agar plates and adding free-living nematodes as bait. Germinating spores, trapping devices, trapped nematodes and retention of their fungal predatory activity was considered as the indicators of viability.

The efficacy of the fungal predatory activity was measured by comparing the mean numbers of recovered larvae from either faecal cultures and grass, in both groups, treated and untreated, in every sampling day based on the following formula:

$$\%\text{Reduction} = (\bar{x}\ \text{Control group} - \bar{x}\ \text{Treated group}/\bar{x}\ \text{Control group}) \times 100$$

Source: Fitz-Aranda et al. (2015).

The means of recovered larvae, either from faecal cultures or from grass were compared between the two groups in each corresponding sampling day throughout the whole experiment, using the *t*-test. Data were analysed using the SAS program (SAS 9.90). The data of epg and numbers of recovered larvae from faecal cultures were log (x + 1) transformed to normalize them before the corresponding analysis.

3. Results

3.1. Duddingtonia flagrans chlamydospore viability

The viability of chlamydospores after being orally administered in calves of treated group is shown in Table 1. No nematophagous fungus in agar plates corresponding to the control group was found. Apart from positive samplings in three calves after four days of the beginning of treatments, no positive animal faeces were found until 12th days after treatment. From 12th day of treatments and until the end of the experiment (day 30^{th}), most samples were positive to